PROCEDURES FOR PREPARATION OF PLASMA

(These procedures supplement the "Regulations Governing Blood Donors, Blood Banks and Plasma Banks" and relate to Section 108 of the Sanitary Code of the City of New York, and were approved by the Board of Health on December 14, 1943, effective January 1, 1944)

1. The method employed for the removal of blood from the donor shall conform to the accepted standards of aseptic surgery.

2. The apparatus used for the removal of the blood and the receiving unit shall be chemically clean and sterile, and shall contain as an anticoagulant either sodium citrate of known pyrogen-free quality dissolved in a pyrogen-free distilled water at least equal to Aqua Injectio, U.S.P. XII, or in a pyrogen-free preservative solution conforming to any of the following or equally satisfactory formulae:

1) Sodium Citrate (dihydric) (3.2%) 2 parts
Dextrose (anhydric) (5.4%) 3 "
Blood 10 "
Reference: "The Survival of Preserved Erythrocytes After Transfusion", Canadian Medical Assn. Journal, 1943.

2) Sodium Citrate (dihydric) (3.2%) 2 parts
Dextrose (anhydric) (5.4%) 13 "
Blood 10 "
Reference: "Studies on Preserved Human Blood. Various Factors Influencing Hemolysis", Journal of the American Medical Association, 1940.

	Percent
3) Sodium Citrate (dihydric)	0.43
Dextrose (anhydric)	4.68
Monobasic sodium phosphate	0.025
Dibasic " "	0.25

This Destrose Buffer Citrate solution is used in the following proportions:

Dextrose Buffer Citrate Solution	3 parts
Blood	2 "

Reference: "The Use of Stored Blood and Plasma in Clinical Practice". Missouri State Medical Society, April 27-28, 1942.

	Grams per liter of solution
4) Sodium Citrate (dihydric)	8.
Dextrose (anhydric)	18.66
Sodium chloride	4.18

This solution is used in the following proportions:

Solution	1 part
Blood	1 part

Reference: "A New Method for the Preparation of Dilute Blood Plasma and the Operation of a Complete Transfusion Service." N.Y. State Journal of Medicine, January 1941.

Of these four formulae, formula #1 is preferred.

3. An acceptable serological test for syphilis shall be made in a qualified laborator on a specimen of blood taken from the donor at the time of bleeding and the blood shall not be used for the production of normal human plasma unless the result of the test is negative.

4. Each bleeding shall be drawn into its own receiving bottle, which shall be the same container used later for separating the plasma from the red cells. Pooling of whole blood before separating the plasma from the red cells is not permitted. It is advisable that within 1 hour and not later than 5 hours after bleeding the blood shall be placed in a cold chamber which shall have a temperature range of 2° to 5° C. (freezing must be avoided). If transportation of the blood before removal of the red cells, from bleeding clinic to processing laboratory, becomes necessary it shall be transported in shipping cases provided with refrigeration sufficient so that (a) if the blood has already been cooled as specified above, the temperature of each individual blood will not be in excess of 10° C. on arrival at the processing laboratory, or (b) if the blood is shipped before its temperature has been reduced to 2° to 5° C. the refrigeration capacity of the shipping case shall be such as to insure continued reduction (the reduction capacity need not extend below 10° C.) of the temperature of each blood during the transportation interval. In either event the blood shall be placed in a cold chamber having a temperature range of 2° to 5° C., immediately upon arrival at the processing laboratory and until required for further processing. The blood may be removed from the cold chamber during the interval required for freeing the

plasma of cells and then shall be returned immediately to the cold chamber. The plasma shall be separated from the cells within 72 hours by centrifugation and within 7 days by sedimentation of the time of drawing the blood from the donor, if collected in a citrate solution, or within 14 days if collected in a pyrogen-free preservative solution in accordance with one of the formulae stated in paragraph 2.

5. It is recommended that all transfers of plasma from one container to another be made in a closed system. A closed system is defined as such an apparatus which will permit nothing to be drawn into the system at any point except the liquid under transfer and the air required for replacement when negative or positive pressure is applied at the proper place. All air for replacement must first pass through a suitable antibacterial filter, except that when the transfer is being made in a closed "sterility room" equipped with mechanical or physical air-sterilizing devices of proven quality, and in operation when the transfer is being made, the air for replacement need not be passed through such a filter.

PREPARATION OF THE PLASMA POOL

6. The supernatant plasma shall be drawn from the bleeding bottle after adequate centrifugation or sedimentation through a closed system into the pool bottle. Only pooled human plasma shall be placed in the final container and for this purpose a minimum of 8 individual plasmas shall constitute a pool. The completed pooled plasma shall contain not more than 50 mgm. of hemoglobin per 100 cc. (See appendix B for a method of determining hemoglobin.)

7. It is recommended that the end of the closed system which is introduced into the plasma be fitted with a glass or metal tube of suitable caliber and that the shaft of this tube be enclosed in a suitable flexible sheath of rubber or other equivalent material. The upper end of the sheath is attached to the rubber tubing where it joins with the above mentioned glass or metal tube and the lower end is fitted around the neck of a glass bell, the dimensions of which are such that the bell will not touch the lip of the bleeding bottle when the rim is resting on the shoulder of the bleeding bottle. When the protective sheath is fully extended,

the above glass or metal tube should reach through the neck of the glass bell and slightly beyond. When in operation the glass bell is placed over the mouth of the plasma-containing bottle so as to rest on the shoulder of this bottle in order that the above glass or metal tube may be inserted into the plasma. The exposed end of the glass or metal tube should be protected by a suitable device so that the plasma will be drawn into the tube from the sides rather than from below.

8. If plasma is to be processed only to the liquid state and dispensed in this form, or if it is intended to be processed to the frozen state without subsequent drying, it is recommended there shall be added to the pool prior to taking the sterility sample a sufficient amount of sterile 50 per cent dextrose solution so as to give a 5 per cent concentration of dextrose in the finished plasma. Dextrose shall not be added to the plasma which is to be shell frozen and subsequently dried.

9. Immediately after withdrawing the sample for the sterility test a sufficient amount of a suitable preservative may be added, except that phenol or a similar compound shall not be considered a suitable preservative. (At the present time the following preservatives and concentrations are considered suitable: For liquid or frozen plasma phenyl mercuric borate 1:15,000 or sodium ethyl mercuri thiosalicylate (Merthiolate) 1:10,000. For dried plasma phenyl mercuric borate 1:50,000 or sodium ethyl mercuri thiosalicylate (Merthiolate) 1:35,000.) The use of a preservative shall not be relied upon for the maintenance of sterility and should not be considered as a substitute for meticulous care in the prevention of contamination of the plasma.

TESTS FOR STERILITY

10. Tests for sterility are required on the plasma before the addition of the preservative. At this point the tests may be made on the plasma from the individual bleedings or on a sample taken from the plasma pool prior to the addition of the preservative. Sterility tests are also required on the finished product as contained in the finished dispensing unit.

11. Test culture medium--The standard culture medium for making the sterility test is designated as "Fluid Thioglycollate Medium" and may be prepared according to two

recognized formulae. (Appendix F) However, experience has shown that of these the Linden formula is the simpler and also more economical and is therefore recommended. (See appendix C)

12. Sterility tests on individual bleedings.--When plasma is to be processed to the liquid or frozen state, the sterility test may be made on the individual bleedings. The following procedure shall be followed: The plasma shall be drawn off into separate individual containers through a closed system and retained in these containers until the sterility test is completed. The test sample shall be withdrawn from these individual plasma containers. The amount of the test sample shall be not less than 5 cc. from each bleeding irrespective of the volume of the blood drawn. This amount shall be cultured at 37° C. for 7 days in one or more portions of thioglycollate medium. If evidence of contamination appears the plasma shall be discarded.

13. When plasma is to be processed to the dried state sterility tests may be made on the individual bleedings. The following procedure shall be followed: The size of the test sample shall be not less than 2.0 cc. from each bleeding irrespective of the total volume of blood drawn. This amount shall be cultured at 37° C. for 48 hours in one or more portions of thioglycollate medium. If evidence of contamination appears the plasma shall be discarded. Since pooling, filling of the final containers, and freezing is required within 72 hours (paragraph 26), pooling of the plasma must follow immediately after the 48 hour reading. However, plasma cultures should be incubated at 37° C. for the full 7 days and a record made as a check on the 48 hour readings.

14. Sterility test on the pool.--When this method of testing for sterility is selected the procedure shall be as follows: For liquid or frozen plasma 2 separate samples shall be withdrawn from the well mixed pool for the sterility test. The size of each sample shall be not less than 20 cc. for each liter of plasma in the pool under test. For the sterility test the entire volume of one of the two samples shall be planted in one or more portions of thioglycollate medium. If evidence of contamination appears the test shall be repeated with

the second sample and if this test also shows the presence of contamination the pool shall be discarded.

15. Plasma intended for drying.--Immediately after pooling 2 separate samples shall be withdrawn from the well mixed pool for sterility testing. The size of each sample shall be not less than 5 cc. for each liter of plasma in the pool under test. For the sterility test the entire volume of one of the two samples withdrawn shall be planted in not less than 10 culture tubes containing adequate volumes of thioglycollate medium. In case contamination appears in 70 percent or more of the tubes on this retest the entire plasma pool shall be discarded. If contamination appears in less than 70 percent of the tubes on the retest the plasma may be processed to the final dried form and then shall be held for a special sterility test as described in paragraph 23.

16. Sterility test on the final containers.--When liquid plasma is being processed a sterility test shall be made on the plasma in a final container, but this test shall not be made before the lapse of at least two weeks since filling the containers from the plasma pool. During this interval the plasma shall have been stored as required under paragraph 40.

17. For the purpose of the sterility test a sample shall be withdrawn from the pool-reservoir at the time of filling the final containers. This sample shall be a pool made up of the first 25 cc. flowing from the plasma-reservoir and the last 25 cc., provided not more than 25 final containers are involved. If the plasma-reservoir is of larger volume, then additional samples shall be prepared on the basis of one additional sample for each 25 final containers or fraction, the sample to be withdrawn from the plasma-reservoir just prior to and immediately following each group of 25 final containers. The sample shall be drawn directly into a separate, empty, final container which has been selected at random from the stock of empty sterile containers.

18. After the lapse of the 2 week storage period (paragraph 16) and after allowing the sample to come in contact with the entire inside surface of the container not less than a 20 cc. portion shall be withdrawn for planting for the sterility

test. The dilution of the plasma in the culture medium shall be such that the preservative contained in the plasma will no longer prevent bacterial growth. (See appendix C) In case contaminations appear in any tube planted, the test may be repeated from the unused portion of the sample, but no lot shall be passed until the final test shows no growth.

19. However, if the lot fails because of growth in the above tests, a retest may be made by selecting at random one of the filled final containers represented by the original sample tested and by carrying out similar sterility tests. The absence of growth on this retest shall negate the previous unsatisfactory sterility tests and the final containers filled between the two portions of the sample showing contamination shall be released as satisfactory.

20. Frozen plasma.--Proceed exactly as directed for liquid plasma (paragraphs 17, 18 and 19) except that the test sample shall be held for the 2 week period as directed in paragraph 16 but in the frozen state as directed in paragraph 29, along with its pool mates, before thawing as directed in paragraph 44. The test sample should be held at room temperature after thawing for 24-48 hours before the cultures are taken.

21. Dried plasma.--The plasma sample for sterility test shall be collected at the time of pooling in the same manner as directed for liquid plasma under paragraph 17. After collection of the sample it shall be subjected to shell freezing and drying under the same conditions of exposure as to time and temperature as is used for final containers. The dried sample shall then be restored to its original liquid volume by the addition of diluent taken from a completed container of diluent as described under paragraph 36.

22. Sampling and testing of this restored plasma shall be carried out exactly as described under paragraphs 17 and 18, except that not less than a 10 cc. portion shall be tested instead of "not less than 20 cc." as required for liquid or frozen plasma.

23. However, if the entire pool falls into the special category of showing contamination, but less than 70 per cent, on the basis of the pool sterility test as described in paragraph 14, a more rigid final sterility test must be made.

For this test a final container of dried plasma shall be taken at random from the lot and dissolved in the prescribed way (paragraph 21), using the amount of diluent necessary to return the plasma to its original volume. Of this restored plasma not less than 2 samples of not less than 50 cc. each shall be cultured in suitable quantities of culture medium (see appendix C). If growth appears in either or both portions the test may be repeated, using for this test a new bottle of plasma. If evidence of contamination is confirmed the entire pool shall be discarded.

THE FINAL CONTAINER

24. Type to be used.--In the processing of plasma either to the liquid, frozen or dried state, the final container shall be filled directly from the pool-reservoir The final container shall be of good quality clear glass closed after filling with an air, moisture and bacteria impermeable seal and shall be constructed to withstand all ordinary handling and shipping hazards without danger of breaking or otherwise impairing the original state. The container shall be equipped so that the necessary connections for the injection of the plasma into the recipient may be attached easily, directly and aseptically to the closure of the plasma container.

25. The final dried product shall contain not more than 1 percent of moisture at any time during the dating period (paragraph 46). "Moisture" is defined as that weight which is lost when the dried plasma is exposed over P_2O_5 in a vacuum chamber. (See appendix E for the method of determining moisture.)

26. Method of filling.--Filling of the final containers when processing to liquid or frozen plasma shall be done not later than 24 hours after completion of the sterility tests on the individual containers, or on the pool. Filling of the final containers, when processing to the dried state, shall be accomplished within such time after the blood is removed from the donor so that the plasma in the container can be brought to the frozen state within 72 hours of the removal of the blood from the donor.

27. The entire pool shall be filled into containers during a single filling operation and this shall be accomplished through a closed system (paragraph 5). The lumen of the filling tube leading to the final container shall be fitted with a filter adequate for the removal of all particles of such coarseness as to be dangerous for intravenous administration. (See appendix D for a description of a suitable filter.)

28. The container into which the plasma is filled from the pool-reservoir shall also be the final container used for dispensing the finished product. At no subsequent stage, as for example during the drying, restoration, or otherwise, shall the plasma be removed from this container except for injection into the recipient.

29. Plasma to be processed to the frozen state shall be placed in the freezing compartment immediately after filling. In order to satisfactorily bring the plasma to the frozen state the properly stoppered containers are placed in a mechanical deep-freeze type of compartment or its equivalent. The temperature of the freezing compartment or device, and its freezing capacity, shall be such that each bottle of liquid plasma placed therein will be brought in its entirety to the frozen state within 6 hours.

30. When plasma is to be processed to the dried state the drying may be begun immediately after shell freezing or the frozen plasma may be stored at a sufficiently low temperature (preferably -18° C. or lower) to satisfactorily hold it in the frozen state to await the outcome of the sterility test or to await a more convenient time for carrying out the drying process. In either event, the final disposition of the entire lot shall depend upon the results of the tests for sterility referred to in paragraphs 21, 22 and 23. Drying shall be accomplished by a method which is not deleterious to the plasma constituents and which will result in a readily soluble product. In general, the finished dried product is of a finer quality if the drying is completed relatively soon after being brought to the frozen state.

31. General instructions.--The final container for the liquid or frozen plasma shall bear a label showing the official name of the product, the amount of citrated plasma present, the amount and kind of preservative present, the amount of diluent added, if any, with its composition, the lot number and if the container label is the outside label it shall also show the expiration date and the name and address of the manufacturer.

32. The final container of dried plasma shall bear a label indicating all of the points in paragraph 31 and in addition the amount of diluent needed to restore the dried material to its original liquid volume. In addition, the label shall carry a statement recommending that the plasma should be used promptly after restoration.

33. It is recommended that either the label or an accompanying circular of instructions contain a statement warning against the danger of overheating the liquid plasma before administration and that when safe warming facilities are not available, or when an emergency exists, it is safe to administer the plasma without preliminary warming.

34. It is recommended that either the label or an accompanying circular of instructions contain a warning as to the danger of injecting the plasma intravenously without the use of a filter in the lumen of the tube leading from the plasma-reservoir to the recipient. (See appendix D.)

35. It is recommended that a sterile and suitable apparatus for injecting the plasma into the recipient accompany each final container of plasma.

36. In the case of dried plasma the label or an accompanying circular shall give full directions for re-dissolving the dried plasma as applicable to the particular type of container used and full directions for getting the restored plasma from the container into the recipient in an aseptic manner.

THE DILUENT FOR DRIED PLASMA

37. A suitable container holding the necessary amount of pyrogen-free, sterile, and otherwise suitable diluent containing 0.1% citricacid shall accompany each

container of dried plasma. This container shall be closed after filling with an air, moisture and bacteria impermeable seal. The quantity of diluent in the container shall be enough to restore the plasma to its original volume. The container shall be equipped so that the necessary connections for the transfer of the diluent to the dried plasma may be attached easily, directly, and aseptically. (See appendix A, pyrogen test.)

38. However, the above requirement shall not be effective if specific requests for the dried plasma without the diluent are made by hospitals, clinics, etc. When dried plasma is dispensed in this manner, the accompanying circular shall give, in addition to other directions, explicit directions and warnings as to the kind of diluent required, the quantity to be used, and any other essential information in order to safeguard the recipient.

DATING AND STORAGE OF PLASMA

39. Liquid plasma.--The expiration date for liquid plasma shall not exceed 2 years from the date of manufacture. The date of manufacture is calculated as the date of bleeding the donor.

40. Liquid plasma shall be stored as near as possible at a constant temperature within the range 15° to 30° C. A statement to this effect shall appear on the label. This temperature range does not necessarily insure the preservation for the entire dating period of all immune bodies which may be present in normal plasma. If these substances are desired it is recommended that recourse be had to dried normal plasma, either liquid or dried normal human serum, or to liquid plasma within 2 months of processing or thawing.

41. Frozen plasma.--The expiration date shall not exceed 3 years from the date of manufacture when kept continuously in the frozen state as specified in paragraph 43. The date of manufacture is calculated as the date of bleeding the donor.

42. Frozen plasma which has been thawed as in paragraph 44 and stored as described in paragraph 40 should be used as soon as possible after thawing.

43. Frozen plasma shall be stored continuously at minus 18° C. or lower until needed.

44. Frozen plasma shall be thawed in the following manner: The bottle of frozen plasma is placed immediately in a constant temperature water bath provided with circulating water, or its equivalent, and maintained at 37° C. As soon as all of the plasma is melted, and its temperature has reached the specified storage range for liquid plasma, the bottle is removed and stored until used as recommended for liquid plasma.

45. It is recommended that frozen plasma be kept in the processing laboratory or other suitable depot and that shipments be made only after thawing as directed in paragraph 44. Provided, however, where either the processing laboratory or the purchaser can assure satisfactory shipment in the frozen state and eventual thawing as described in paragraph 44, this may be done.

46. Dried plasma.--The expiration date for dried plasma shall not exceed 5 years from the date of manufacture provided the moisture at all times is less than 1 percent as determined by the method given in appendix E. The date of manufacture is calculated as the date of bleeding the donor.

47. Dried plasma may be stored at the prevailing temperature provided this does not exceed that designated as excessive heat (over 49° C.) by U.S. Pharmacopoeia XII. However, it is recommended that whenever possible it should be kept in a cool place. A statement with regard to storage shall appear on the label.

APPENDIX A

Pyrogen Test (Paragraph 2)

Test animal.--Use healthy rabbits weighing 1000 gm. or more which have been maintained for at least 1 week on a uniform diet and have not lost weight. Test the thermometer to determine the time required to reach maximum temperature. If the animals have not been previously used for such tests, take four rectal temperature readings on each of the animals at 2-hour intervals 1 to 3 days before use. Insert the thermometer beyond the internal sphincter, and allow it to remain sufficient time to reach maximum temperature, but in no case less than 90 seconds, before the

reading is recorded. Discard those animals with a temperature in excess of 39.8° C. On the day of the test take a control temperature reading before the injection of the test material. Animals may be used for the test and in subsequent tests after a rest period of not less than 2 days, provided the control temperature reading taken on the day of the test does not exceed 39.8° C. The reading taken on the test day constitutes the normal temperature of the test animal from which a subsequent rise due to the injection of the test material is calculated. Keep test animals in individual cages protected from disturbances likely to cause excitement. Exercise particular care to avoid exciting the animals on the day of taking the control temperatures and on the test day. Withhold food from any animal used, beginning 1 hour before the first temperature reading, and permit no food until the day's record is completed. Free access to water is allowed. Keep the animals at uniform temperatures (±5° C.) during the control and test period. They should preferably be housed in quarters maintained at constant temperature and humidity.

Conduct of the Test.--Warm the product to be tested to approximately 37° C. and inject 10 cc. per kgm. of rabbit, intravenously through the marginal ear vein within 15 minutes subsequent to the control temperature reading on the day of the test. Record the temperature 1 hour subsequent to the injection and each hour thereafter until three recordings have been made. Syringes and needles used for these injections must have been treated to render them pyrogen-free and then sterilized. Not less than 5 rabbits shall be used for each test and the test shall be considered positive if three or more of the 5 animals show an individual rise in temperature of 0.6° C. or more above the normal established for each of these animals. If only 1 or 2 of the 5 animals show a positive response the test must be repeated on a second group of 5 additional animals. The test shall be considered positive if 2 of the second group of 5 animals show an individual rise in temperature of 0.6° C. or more above the normal established for these animals.

APPENDIX B

Methods for the Determination of Hemoglobin (Paragraph 6)

1. The determination shall be considered sufficiently accurate if a colorimetric comparison be made with a standard prepared from hemolyzed blood from a person whose blood hemoglobin content has been determined previously in terms of grams of hemoglobin. The standard is prepared as a dilution of hemolyzed blood in plasma which is free of all visible trace of hemoglobin color. Hemolysis of the blood is effected by first diluting the blood 1 to 20 with distilled water and waiting until hemolysis is complete.

2. The blood hemoglobin in the person serving as the source of hemoglobin supply shall be determined by a method at least as accurate as can be made by a Sahli apparatus equipped with permanent color standards. These color standards shall have been calibrated during the course of preparation by the manufacturer, using the Van Slyke oxygen capacity method, the Wong iron method, or a method recognized as the equivalent. (Ref.--Am. J. Clin. Path., v. 3, p. 85; v. 4, p.354)

3. For an accurate quantitative determination of the hemoglobin content of the pooled plasma either of the following methods may be employed, or one equally accurate:

Arch. Int. Med., v. 64, p. 1271 (Dec., 1939)

J. Biol. Chem., v. 92, p. 589, 1931).

APPENDIX C

Fluid Thioglycollate Medium for the Sterility Test (Paragraph 11)

1. This culture medium will be acceptable if prepared by either of the two methods given below, provided that if Method B or the designated dehydrated variations of Methods A or B are used the growth promoting qualities for the contaminating organisms are no less than is the case if cultured in a medium prepared by Method A. Methods A and B are recognized as equally satisfactory but Method B is recommended as easier to prepare and more economical. The completely prepared

media which is available is preferred since that product has already had cultural tests and is otherwise known to be satisfactory.

2. a. Method A. - Fluid Thioglycollate Medium (Brewer): The broth base for this medium is prepared as follows (Changes in the order of compounding the broth or in the meat base are permitted provided the growth-promoting qualities remain at least equal to those obtained by the method as described herein):

Ground fresh beef (freed of fat)	500 grams
Sodium chloride	5 "
Dipotassium phosphate (K_2HPO4)	2 "
Peptone	10 "
Distilled water	1000 cc.

Put the ground meat into the distilled water, mix thoroughly and allow to stand at 5°C. for 24 hours. Collect the liquid by straining through cloth, then heat for 1 hour in streaming steam followed by 30 minutes at 15 pounds pressure (121°C.) in the autoclave. Filter while hot through moistened filter paper and make up to the original volume. Add the remaining ingredients and stir until solution is completed. Adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7.5 ± 0.1, in the completed broth. Heat in streaming steam for 30 minutes, clear by filtration, fill into suitable containers and sterilize in the autoclave at 15 to 17 pounds pressure (121° to 123°C.) for 20 minutes.

To 1000 cc. of this stock broth add the following ingredients:

	Grams
Dextrose (anhydrous)	5.0
Sodium thioglycollate	1.0
Agar (Less than 15% moisture by weight)	0.5
Methylene blue (Use 1 cc. of a 1:500 solution)	0.002

Add the agar to the cold broth, mix well and heat gradually to the boiling point. Cool to approximately 80°C. and add the remaining ingredients. Stir until solution is completed and the ingredients are uniformly distributed. Adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7.5, ± 0.1, in the completed and sterile medium. Distribute in final containers of the desired size and sterilize in the autoclave for 18 to 20 minutes at 15 to 17 pounds pressure (121°C, to 123°C.).

2. b. A dehydrated medium consisting of the essential materials contained in the above formula is commercially available. Such a medium is acceptable provided it meets the various requirements herein specified for a thioglycollate medium.

3. a. Method B - Fluid Thioglycollate Medium (Linden):

Peptone	20.0 grams
Dextrose (anhydrous)	5.0 "
Yeast extract	2.0 "
Sodium thioglycollate	1.0 "
Sodium chloride	5.0 "
Agar (Less than 15% moisture by weight)	0.5 "
Dipotassium phosphate (K_2HPO_4)	2.5 "
Distilled water	1000.0 cc.
0.2% solution of Methylene blue (cert.)	1.0 "

Dissolve the agar in half the volume of distilled water by boiling or heating in the Arnold. Dissolve the remaining ingredients, except the methylene blue, in the remaining water with the aid of heat. Now mix the two portions, adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7.5, $\pm$ 0.1, in the completed and sterilized medium. Filter clear while hot and add the methylene blue solution. Distribute into final containers of the desired size and sterilize in the autoclave for 18-20 minutes at 15-17 pounds pressure (121°C. to 123°C.).

3. b. A medium may be prepared as a pre-mixed, dehydrated stock of the essential ingredients contained in method B. formula. Such a medium is acceptable provided it meets the various requirements herein specified for a thioglycollate medium as defined under Method A.

4. CAUTION: Use distilled water throughout and avoid the addition of any calcium salt.

Sodium thioglycollate is a white or faintly yellow crystalline powder with slight odor. Deterioration is indicated by a change of color to yellow or yellowish-brown, and is accompanied by a stronger odor which ultimately becomes definitely that of carbon disulfide. Sodium thioglycollate which shows deterioration must not be used.

5. After removal of the final containers from the autoclave, cool to below 25°C. in order to set the agar. Store at 15°C. to 30°C., avoiding excessive light (storage at low temperatures increases absorption of atmospheric oxygen). If more than 20% of the uppermost portion of the medium has changed to a green color, it is not suitable for use. Under such circumstance one reheating in the Arnold or water bath is permissible in order to drive off the absorbed oxygen. A final reaction of pH 7.5 is interpreted to allow a $\pm$ reaction of pH 0.1 at the

time of manufacture.

6. Each lot of medium shall be tested for its growth-promoting qualities by a trial run, using as the inoculum one or more delicately growing bacteria.

At the end of the incubation period used for the sterility test less than 50% of the medium in each tube shall have changed from the color of the fresh medium to a green color.

7. This medium permits the growth of both aerobic and anaerobic organisms in open containers. The type of container and the amount of medium used is dependent upon the use to which it is to be put. Tall, slender containers are recommended and in each instance a sufficient amount of medium shall be used so as to provide ample dilution of any preservative contained in the substance to be tested for sterility. This medium is particularly effective in neutralizing the growth-inhibiting action of mercurials. In each instance the inoculum should be mixed thoroughly into the medium.

8. When smears are to be made from cultures in this medium it is recommended that they be fixed with methyl alcohol instead of heat since this gives a clearer background.

APPENDIX D

A Filter Adequate for Removal of Particulate Matter (Paragraph 27)

1. A filter adequate for the removal of all particles of such coarseness as to be dangerous for intravenous administration must be placed in the tube through which the plasma flows from the plasma-pool reservoir to the final container (paragraph 27). In addition, it must be placed in the lumen of the tube provided with each unit of either finished liquid, frozen or dried plasma (35) for the purpose of transferring the plasma from the container to the blood stream of the recipient. Further, if such equipment is not provided the label on the final container (paragraphs 34 and 38), or the accompanying direction circular for administration of the plasma, must bear a warning that such filter must be used.

2. A metal filter shall be considered adequate provided it is no less effective than is obtained by a stainless steel filter when prepared as follows: The metal filter consists of a cylindrical tube 1 to 1⅛ inches in length, made of stainless steel screen or monel metal screen of 160-200 mesh. It should be 3¼ to 4 mm. in diameter closed at one end. The other end inserts into a stainless steel metal ring 1 mm. in thickness. This is inserted in a glass tube with the metal ring uppermost towards the dispensing bottle.

APPENDIX E

A method for the Determination of Residual Moisture (Paragraph 25)

1. The amount of residual moisture remaining in plasma which is labeled as a dried, or desiccated, product shall contain not more than 1 per cent moisture when determined by the following method: "Expose a 1 to 2 gram sample of the plasma, evenly distributed in a weighingbottle not less than 60 mm. in diameter, in a vacuum desiccator at less than 1 mm. pressure and over fresh phosphorus pentoxid at room temperature until the weight remains constant to the third decimal." In removing the sample to the weighing bottle, it is important to avoid unnecessary exposure of the dried plasma to the air, particularly if the moisture content of the atmosphere is relatively high.